Frequency of Sexual Reproduction by *Mycosphaerella graminicola* on Partially Resistant Wheat Cultivars

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ABSTRACT

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The frequency of sexual reproduction has a profound effect on the population structure and the adaptive potential of a facultatively sexual parasite. Little is known about the relationship of quantitative host resistance to the frequency of sex in pathogens. We sampled over 5,000 fungal fruiting bodies from eight different wheat cultivars over a 3-year period. The cultivars possessed varying degrees of susceptibility to *Mycosphaerella graminicola*, a facultatively sexual pathogen that is heterothallic and bipolar. The fruiting bodies were classified as *M. graminicola* pycnidia or ascocarps (asexual and sexual fruiting bodies, respectively), other identifiable fungi, or unidentified. In all 3 years, area under the disease progress curve (AUDPC) explained a significant proportion of the variation in ascocarps as a percentage of *M. graminicola* fruiting bodies ($P < 0.0005$). The mean percentage of *M. graminicola* ascocarps from all cultivars was 63% in 1998, when the epidemic was intense, and 14% in 1999, a year of low disease levels. In 2000, samples were taken at 7-day intervals from 6 June to 27 June from two cultivars with substantially different AUDPCs (788 and 2,185 percentage-days). The less diseased cultivar yielded its first *M. graminicola* ascocarps 1 week later than the more diseased cultivar, and respective means of ascocarps as a percentage of *M. graminicola* fruiting bodies across sampling dates were 20.2 and 59.3%. The ratio of sexual to asexual reproduction by *M. graminicola* is likely to be strongly conditioned by infection density.

Additional keywords: microconidia, micropycnidiospores, *Phaeosphaeria nodorum*, Red Queen hypothesis, Septoria tritici blotch, *Triticum aestivum*.

As an evolutionary strategy, sexual reproduction can increase pathogenic fitness in three ways. First, it can generate new combinations of alleles (new genotypes) through independent assortment that have higher fitness as a result of interactions among alleles at different loci (19,27,32). Second, through intragenic recombination it can create new alleles (35), possibly at loci that affect pathogenicity (6). Third, sexual reproduction may free beneficial alleles from disadvantageous alleles at other loci and prevent accumulation of low-fitness alleles in a single genetic background (27,28,32,36). Thus, sex can increase the effectiveness of selection and facilitate adaptation by the pathogen population to host defenses or chemical pesticides (4,36).

Experimental evidence indicates that there is a trade-off between sexual and asexual reproduction in many fungal species, and that the balance is influenced by several genetic and environmental factors (8). Different ecological roles for sexual and asexual propagules may help to maintain recombination in the life cycle of organisms that are facultatively sexual, as are numerous plant-pathogenic fungi (7,33). The relative contributions of sexual and asexual reproduction have significant effects on pathogen population structure (1,28), and have been investigated using direct and indirect techniques for several plant pathosystems (5, 30,34,39).

Whether facultative sexual reproduction by a pathogen is more frequent on resistant or susceptible hosts is a matter with important epidemiological and disease management consequences (18). Little empirical evidence is available to shed light on this issue. A study of potato cultivars with different levels of race-nonspecific resistance to *Phytophthora infestans* indicated that oospore formation was highest in cultivars with intermediate levels of resistance, perhaps because susceptible cultivars were destroyed too quickly to allow oospore formation (20). Gemmill et al. (18) reported that facultative sexual reproduction by parasitic nematodes was higher when challenged by a genotype-specific immune response in rats than in the absence of the immune response. They suggested that sex in pathogens might be an adaptation to combat facultative antiparasite defenses. This would essentially be the converse of the Red Queen hypothesis, which holds that sexual reproduction is maintained in hosts as a response to cyclical changes in selection pressure caused by pathogens over time (4,10). A theory that accounts for sexual reproduction in facultatively sexual organisms as a response to stress (2) would also lead one to expect higher levels of pathogen sexual reproduction on more resistant hosts.

Mycosphaerella graminicola, a fungus that is probably bipolar and heterothallic (26), is a facultatively sexual pathogen of wheat (*Triticum aestivum*). This fungus causes the increasingly damaging foliar disease Septoria tritici blotch. A good deal is already known about the sexual stage of *M. graminicola* in winter bread wheat. Ascospores, the sexual propagules, are born in pseudothecia (ascocarps). Ascospores are not only the primary source of inoculum that initiates epidemics (37,38) but are also produced under at least some conditions throughout the year (21,26). The sexual stage has a major impact on the genetic structure of *M. graminicola* populations (9,31), which are characterized by high levels of gene and genotypic diversity (30).

The role of sexual reproduction in disease progress, and the environmental and host factors that influence the degree of sexual reproduction, are still uncertain. Kema et al. (26) reported completion of a sexual cycle on a susceptible cultivar in 5 weeks, and suggested that *M. graminicola* could complete several sexual cycles per season. Eriksen et al. (16) predicted on the basis of

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mathematical modeling that, while ascospores should have little impact on epidemic progress, the proportion of all pycnidia (asexual fruiting bodies) that were sexual descendants could be as high as 20 to 30% by the end of the growing season. Similarly, Zhan et al. (41,42) estimated 21% sexual recombinants in a sample of 600 isolates gathered from field plots at the end of a growing season and analyzed with molecular marker data.

To the best of our knowledge, to date, no published research about the role of sexual reproduction in this disease system has specifically taken into account differences in host susceptibility. We sampled fruiting bodies from wheat cultivars with varying levels of resistance to *M. graminicola*. Our purpose was to better understand the relationship between degree of host resistance and the ratio of sexual to asexual reproduction.

MATERIALS AND METHODS

Field plot establishment. Field plots of winter bread wheat cultivars with varying levels of partial resistance to *M. graminicola* were established in 1998, 1999, and 2000 at the Oregon State University Botany and Plant Pathology Field Laboratory in Corvallis, OR. Of the six cultivars used in the first experimental year, three (cvs. Stephens, Madsen, and MacVicar) were commonly grown in western Oregon, where the experiment took place. The other three cultivars, not commonly grown in this region, were chosen to provide a balance between moderately resistant and susceptible cultivars. The moderately resistant cultivars had expressed approximately one-half of the level of disease found on the susceptible cultivars in western Oregon (J. A. DiLeone and S. M. Coakley, *unpublished data*). Days to heading, which is moderately correlated with resistance to *M. graminicola* (14), also varied among cultivars (Table 1). Heights of the six cultivars were unassociated with susceptibility to *M. graminicola*.

In 1999, cvs. Gene and Foote were added to the experiment in order to enlarge the sample and because 'Foote' was both moderately resistant and had a similar maturity date to those of susceptible cvs. Stephens, W-301, and MacVicar (Table 1). At its release in 1992, 'Gene' was highly resistant to *M. graminicola*, but that resistance had largely disintegrated by 1995, and specific interactions between 'Gene' and the pathogen have been demonstrated (13). 'Gene' matures approximately 10 days earlier than the other susceptible cultivars in the experiment. In 2000, only two cultivars ('Gene' and 'Stephens') were included in the experiment because the number of dates on which leaves were sampled was increased from one to four.

Each of the four plots of each treatment occupied two adjacent 1.5- × 6.1-m planting units. Plots of wheat and barley were alternated in a checkerboard pattern so that the barley provided a buffer between the wheat plots. Plots were planted on 20 October 1997, 7 October 1998, and 12 October 1999. Fertilization, planting, and weed control were all conducted according to standard commercial practices in the area.

Fungicide applications. Reduction of disease levels on susceptible cultivars was accomplished by applying the nonsystemic, protectant fungicide chlorothalonil (Bravo 720, 2.72 kg of chlorothalonil per liter, 54% a.i.). In 1998, the concentration was 3.7 ml

TABLE 1. Percentages of *Mycosphaerella graminicola*, *Phaeosphaeria nodorum*, and unidentified fruiting bodies sampled at the end of three growing seasons from four replicated field plots of wheat cultivars with differing levels of susceptibility to Septoria diseases

			M. graminicola				
Year[a]	Treatment[b]	Days to heading[c]	Sexual (% of total)[d]	Asexual (% of total)[d]	Sexual (% of *M. graminicola*)[e]	*Phaeosphaeria nodorum* (% of total)[f]	Unidentified (% of total)[g]
1998	Cashup	145.0	23.8	11.3	67.9	3.1	61.9
	Lewjain	147.5	2.8	60.6	4.4	7.0	29.6
	MacVicar	139.0	27.7	4.6	85.7	6.9	60.7
	Madsen	140.5	39.4	4.7	89.3	1.8	54.1
	Stephens	138.0	45.1	3.4	92.9	3.4	48.1
	W-301	138.5	30.2	4.5	87.1	5.0	60.3
	Steph-spray[h]	…	3.8	19.2	16.7	1.9	75.0
Mean			24.7	15.5	63.4	4.2	55.7
1999	Cashup	145.0	0.7	41.7	1.6	0.0	57.6
	Foote	138.5	0.0	44.4	0.0	6.5	49.1
	Gene	128.0	5.2	15.1	25.5	0.9	78.9
	Lewjain	147.5	0.0	70.5	0.0	0.0	29.5
	MacVicar	139.0	4.7	23.0	16.9	0.0	72.3
	Madsen	140.5	0.4	31.6	1.4	0.0	68.0
	Stephens	138.0	11.0	23.9	31.5	0.5	64.6
	W-301	138.5	4.4	24.5	15.3	0.5	70.6
	Steph-low[h]	…	5.7	31.6	15.4	0.0	62.7
	Steph-high[h]	…	12.4	37.3	25.0	0.6	49.7
	W301-low[h]	…	1.8	52.4	3.3	0.0	45.9
	W301-high[h]	…	10.9	31.4	25.9	0.0	57.7
Mean			4.8	35.6	13.5	0.7	58.8
2000	Gene	128.0	5.2	10.9	32.1	56.3	27.6
	Stephens	138.0	23.4	8.7	72.9	16.8	51.1
Mean			14.3	9.8	52.5	36.6	39.4

[a] Fruiting bodies sampled from leaves collected on 2 July 1998, 29 June 1999, and 27 June 2000.
[b] 'Cashup', 'Foote', 'Lewjain', and 'Madsen' were moderately resistant to *M. graminicola*. 'MacVicar', 'Stephens', and 'W-301' were susceptible. 'Gene' was susceptible in 1998 to 1999 and moderately resistant in 2000.
[c] Julian days in Corvallis, OR, averaged for 1994 and 1995, except 'Foote' for which the mean of Julian days to heading in 1997 and 1998 was adjusted by the average differences between the other cultivars' readings in the two pairs of years (22–25).
[d] *M. graminicola* pycnidia or pseudothecia as a percentage of all fruiting bodies sampled.
[e] Pseudothecia as a percentage of *M. graminicola* pseudothecia and pycnidia.
[f] Obvious *Phaeosphaeria nodorum* lesions were excluded when selecting leaf segments for fruiting body sampling in order to enrich samples for *M. graminicola* fruiting bodies.
[g] Fruiting bodies of fungi recognizable as other than *M. graminicola* and *Phaeosphaeria nodorum* ranged from 0.0 to 8.3%.
[h] In 1998, chlorothalonil was applied to each plot at a rate of 0.84 liter per plot and a dosage of 3.7 ml of chlorothalonil per liter of water seven times during the growing season. In 1999, the dosages were 1.8 ml/liter (high) and 0.88 ml/liter (low) and five applications were made during the growing season.

of Bravo per liter of water, approximately 80% of manufacturer's recommended strength, with the surfactant Triton B added at the rate of 0.176 ml/liter. Applications were made to plots of cv. Stephens at a rate of 0.84 liter per plot (468 liters/ha) seven times at intervals of 2.5 to 5 weeks, depending on the growth rate of the wheat, from 10 December to 22 May.

In the second year of the experiment, lower fungicide dosages of 1.8 and 0.88 ml/liter were utilized in order to effect more moderate disease reductions. The fungicide was applied only five times at approximately 3-week intervals between late January and late April, and plots of a second susceptible cultivar, 'W-301', were also sprayed.

Disease measurements. Visual assessments of percent diseased leaf area were conducted by two assessors for each plot on a whole-canopy basis eight times at 10- to 27-day intervals between 6 February and 15 June 1998, 17 February and 17 June 1999, and 18 February and 6 June 2000.

Leaf sampling. In 1998 and 1999, leaf sampling took place on 2 July and 29 June, respectively. One F-4 leaf (the fourth leaf below the flag leaf) was randomly selected from each of five rows of each replicate plot of each treatment, excluding the outermost rows to avoid edge effects, and the leaves were bagged by replicate. Leaves were collected without regard for the extent of infection with *M. graminicola*. In 1999, F-4 leaves were also collected in the same manner on 26 May from all replicates of 'Gene' and 'Stephens' in order to examine fruiting bodies present prior to the end of the growing season. In 2000, F-4 leaves were collected separately by replicate from plots of 'Gene' and 'Stephens' four times at 7-day intervals starting 6 June and ending 27 June.

Fruiting body sampling and identification. Fruiting bodies were sampled from each field replicate as follows: approximately 7 to 8 cm of leaf tissue with a high density of fruiting bodies was frozen approximately 30 s in liquid nitrogen, and ground for 10 to 15 s into a semifine powder with a mortar and pestle. Working with a fine-pointed needle under a dissecting microscope, 30 to 70 fruiting bodies were selected at random from the powder, with the number selected depending on availability. The exception in sample size was from the 'Stephens' sprayed treatment in 1998, from which a combined total of only 52 fruiting bodies could be recovered from leaves collected in all four field replicates due to the small number of lesions on those leaves.

A total of 5,454 fruiting bodies were examined for the 3 years: 1,154 from 1998, 2,841 from 1999, and 1,459 from 2000. Leaving aside the 'Stephens' high-spray treatment in 1998, a mean of 195 fruiting bodies was sampled from each host on each sampling date in each year across all four field replicates, with sample sizes ranging from 137 to 290 fruiting bodies. The 26 May 1999 samples from cvs. Gene and Stephens contained no *M. graminicola* pseudothecia, and those data are not included in the presentation of results.

Fruiting bodies were sorted and examined by size because previous observations had suggested that larger fruiting bodies tended to be *M. graminicola* pycnidia. Each fruiting body was assigned by eye to one of three size categories (small, medium, and large), such that there were roughly equal numbers of fruiting bodies in each category. Members of a single size category were placed individually on a microscope slide in a small drop of lactophenol blue stain. The fruiting bodies were squashed gently under a coverslip and allowed to absorb stain overnight.

Fruiting bodies were identified under a compound light microscope at ×400 magnification. Each fruiting body was classified as one of the following: *M. graminicola* ascocarp (pseudothecium), *M. graminicola* pycnidium, *Phaeosphaeria nodorum* ascocarp (pseudothecium), *Phaeosphaeria nodorum* pycnidium, or unidentified. Fruiting bodies were only determined to be ascocarps or pycnidia of *M. graminicola* or *Phaeosphaeria nodorum* if clearly recognizable asci, ascospores, or pycnidiospores were present. Asci, ascospores, and macro- and micropycnidiospores of the two genera were distinguished according to the descriptions of Eyal et al. (17), and separate tallies were kept of pycnidia containing macropycnidiospores, micropycnidiospores, or both.

Statistical methods. Logistic regression by the SAS GENMOD procedure (SAS Institute, Cary, NC) was used to assess the effects of the explanatory variables of field replicate, size, and area under disease progress curve (AUDPC) on the dichotomous response variable sexual/asexual for identified *M. graminicola* fruiting bodies. Logistic regression analysis, also known as logit analysis, is a form of multiple regression that is preferable to ordinary linear regression when the dependent variable is dichotomous (binary) (SAS Institute). In the logit model, which is estimated by maximum likelihood, the dependent variable is the natural logarithm of the odds of an event, and the independent variables can be either categorical or quantitative. Our data were fit to the logistic regression model: $\ln(\text{odds of a pseudothecium}) = \alpha + \beta_1\text{Rep} + \beta_2\text{Size} + \beta_3\text{AUDPC} + \beta_4\text{Size}\times\text{AUDPC} + \text{error}$, where Rep is the field replicate (there were four), Size is the fruiting body size (small, medium, or large), and AUDPC is the continuous variable of area under the disease progress curve that reflected host susceptibility.

Because goodness-of-fit criteria provided by the GENMOD procedure indicated variation other than binomial variation in the data, a scaling option (d-scale) was used that multiplies the binomial variance by a constant and performs F tests rather than χ^2 tests. Thus, standard errors increased by a factor of two, making it harder to achieve significance.

Logistic regression was also used to assess the effects of the explanatory variables rep, size, and AUDPC on a dependent dichotomous variable indicating the presence or absence of micropycnidiospores. Although pycnidia were originally scored as having macropycnidiospores, micropycnidiospores, or both, for this analysis, the latter two categories were combined. The d-scale option described previously was used.

TABLE 2. Significance of explanatory variables from logistic regression analysis, with response variable being the pseudothecial (sexual) proportion of all identified *Mycosphaerella graminicola* fruiting bodies sampled in 1998, 1999, and 2000[a]

Parameter	df	*F* value	*P* value
1998			
Rep	3	4.08	0.0066
Size[b]	2	2.97	0.0513
AUDPC[c]	1	115.05	<0.0001
Size ×AUDPC	2	2.77	0.0624
1999			
Rep	3	2.61	0.0493
Size[b]	2	2.54	0.0792
AUDPC[c]	1	43.93	<0.0001
Size × AUDPC	2	0.07	0.9284
2000			
Rep	3	2.52	0.0560
Date[d]	3	10.66	<0.0001
Size[b]	2	3.19	0.0410
AUDPC[c]	1	12.65	0.0004
Size × AUDPC	2	0.13	0.8762

[a] Regression equation: $\ln(\text{odds of a pseudothecium}) = \alpha + \beta_1\text{Rep} + \beta_2\text{Size} + \beta_3\text{AUDPC} + \beta_4\text{Size} \times \text{AUDPC} + \text{error}$, where Rep is the field replicate (there were four), Size is the fruiting body size (small, medium, or large), and AUDPC is the continuous variable of area under the disease progress curve that reflected host susceptibility.

[b] Before staining and identification, fruiting bodies were classified by eye as small, medium, or large.

[c] Area under the disease progress curve was a continuous variable, calculated from visual assessments of percent diseased leaf area conducted by two assessors for each plot on a whole-canopy basis eight times at 10- to 27-day intervals between 6 February and 15 June 1998, 17 February and 17 June 1999, and 18 February and 6 June 2000.

[d] In 2000, F-4 leaves were collected at 7-day intervals starting 6 June and ending 27 June.

Correlation analysis was performed in SAS to assess the strength of the linear relationships between AUDPC and (i) pseudothecia as a percentage of *M. graminicola* fruiting bodies, and (ii) *M. graminicola* pseudothecia as a percentage of all fruiting bodies sampled. The correlation was also assessed between the percentage of unidentified fruiting bodies and percentages in the total sample of *M. graminicola* pycnidia and pseudothecia, respectively.

When comparing the years 1998 and 1999 for the effects of cultivar susceptibility on pseudothecial percentages, mid-June disease severity was used rather than AUDPC as the measure of susceptibility. This was because the shapes of the disease progress curves from the 2 years differed, rendering the comparison of area under them misleading, whereas for within-year comparisons, AUDPC was preferable due to lower error.

RESULTS

In all 3 years, AUDPC was predictive of the percentage of *M. graminicola* fruiting bodies that were ascocarps (Table 2; Fig. 1), with $P < 0.0005$ by logistic regression. Correlation analysis indicated a positive relationship between AUDPC and ascocarps as a percentage of *M. graminicola* fruiting bodies ($r = 0.90$ and $P = 0.005$ in 1998, $r = 0.79$ and $P = 0.002$ in 1999). If the 1999 sprayed treatments were omitted from the analysis, the association became stronger in that year ($r = 0.93$; $P = 0.0009$). There was also a positive relationship between AUDPC and *M. graminicola* ascocarps as a percentage of all sampled fruiting bodies ($r = 0.84$ and $P = 0.017$ in 1998, $r = 0.60$ and $P = 0.040$ in 1999). From logistic regression, a one-unit increase in AUDPC was associated with increases in the odds of an identified *M. graminicola* fruiting body being an ascocarp of 0.46, 0.15, and 1.42% for the 3 years, respectively.

Mean epidemic intensity and mean pseudothecial percentages varied among the years. In 1998, the epidemic developed consistently and strongly throughout the season. In 1999, there were relatively low levels of disease due to a dry late spring. In 2000, disease increased slowly in the early season but rapidly in May and June. Both mean late-season disease severities and mean pseudothecial percentages were greater in 1998 than in 1999 (Fig. 2). Means of pseudothecia as a percentage of *M. graminicola* fruiting bodies were 63.4% in 1998, 13.5% in 1999, and 52.5% in 2000 (Table 1).

In 1998, the application of fungicide reduced both disease severity and percent pseudothecia on 'Stephens' to near the levels on 'Lewjain', the most resistant cultivar (Fig. 1). In 1999, when the high-spray dosage was half that applied in 1998, high and low dosages resulted in similar levels of disease reduction: 19.0, 19.2, 15.2, and 17.3% in the 'Stephens' low-, 'Stephens' high-, 'W-301' low-, and 'W-301' high-spray plots, respectively. Both fungicide dosages reduced pseudothecia as a percentage of *M. graminicola* fruiting bodies on 'Stephens', but only the low dosage did so on 'W-301' (Table 1).

In 2000, pseudothecia constituted a lower percentage of *M. graminicola* fruiting bodies on each sampling date and averaged over all sampling dates on 'Gene' than on 'Stephens' (Fig. 3; means of 20.2 and 59.3%, respectively). In that year, AUDPCs were 788 and 2,185 percentage-days for 'Gene' and 'Stephens', respectively.

While *Phaeosphaeria nodorum* fruiting bodies were absent or at very low percentages on all cultivars in 1998 and 1999, they comprised 56.3 and 15.9% of the fruiting bodies sampled from 'Gene' and 'Stephens', respectively, at an equivalent point in the 2000 growing season (Table 1). In 2000, *Phaeosphaeria nodorum* fruiting bodies increased from 7.4 to 56.3% of all fruiting bodies sampled on 'Gene' between 6 June and 27 June, and increased from 10.5 to 33.7% of all fruiting bodies sampled from 'Stephens' between 6 June and 20 June before dropping again to 16.9% of all fruiting bodies on 27 June. Probably due to competition with *Phaeosphaeria nodorum*, only 24.1% of sampled fruiting bodies were identified as *M. graminicola* in 2000. In that year, 19.6% of fruiting bodies from the two cultivars were *M. graminicola* pseudothecia and 9.8% were pycnidia.

The mean frequency with which *M. graminicola* pycnidia contained some or exclusively micropycnidiospores was 27.7% in 1998 and 56.6% in 1999 (Table 3). In 2000, when only two hosts were sampled, the mean frequency of micropycnidiospores was 30.4%. From logistic regression, no relationship of AUDPC to the presence of micropycnidiospores could be inferred because there were significant rep×AUDPC interactions in both years ($P = 0.0061$ and 0.029 in 1998 and 1999, respectively). In 1998, fruiting body size had no significant effect on the presence or absence of micropycnidiospores ($P = 0.21$). In 1999, size was significant ($P = 0.0004$), with the odds of finding micropycnidiospores in large fruiting bodies only 39% of the odds of finding them in medium or small fruiting bodies.

Among *M. graminicola* fruiting bodies, smaller size was generally associated with a lower frequency of pseudothecia (Table 2). In 1998, the relationship of size to proportion of pseudothecia varied with AUDPC: higher pseudothecial proportions were found

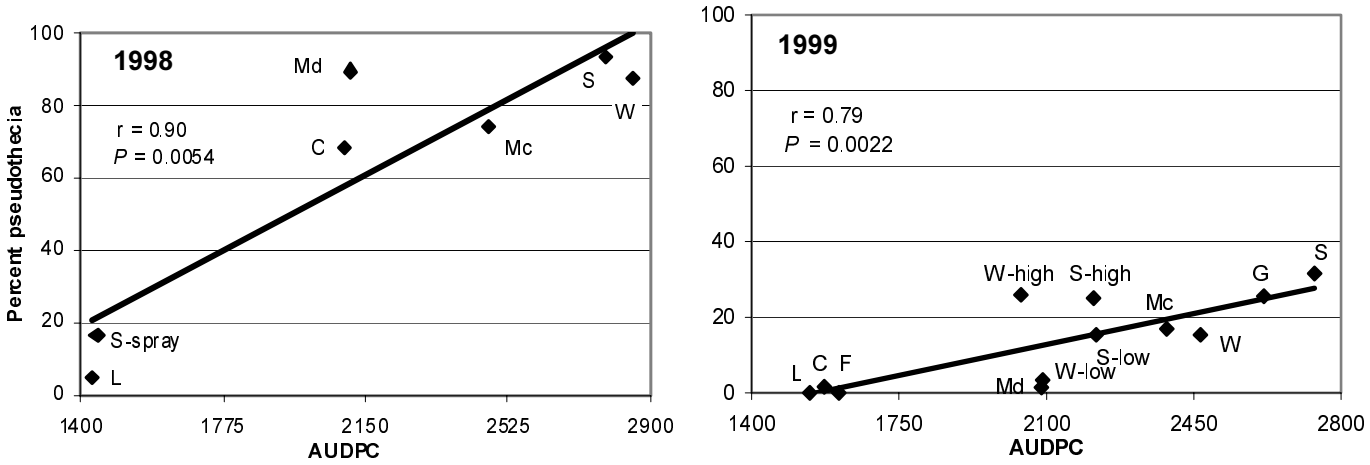

Fig. 1. Regression of pseudothecia as a percentage of all *Mycosphaerella graminicola* fruiting bodies on area under the disease progress curve (AUDPC) assessed in four replicated field plots of each host. Hosts: C = Cashup, F = Foote, G = Gene, L = Lewjain, Mc = MacVicar, Md = Madsen, S = Stephens, and W = W-301. Low = low-spray; high = high-spray. To reduce epidemic development, 0.84 liter of chlorothalonil was applied to each plot at a dosage of 3.7 ml/liter of water seven times during the 1998 growing season. During the 1999 growing season, five applications were made with dosages of 1.8 ml/liter (high) and 0.88 ml/liter (low).

among large fruiting bodies for higher AUDPCs and among medium-sized fruiting bodies for lower AUDPCs. In 1999, higher pseudothecial proportions were found among larger fruiting bodies, although the effect of size was only significant at P = 0.079. Across all sampling dates in 2000, size was significant at P = 0.041 and higher pseudothecial proportions were found among larger fruiting bodies. In general, fruiting bodies seemed to be smaller on moderately resistant than on susceptible cultivars.

Correlation analysis indicated that in both 1998 and 1999 there was a negative association between the percentages of *M. graminicola* pycnidia and unidentified fruiting bodies (r = –0.70 and P = 0.082 in 1998, r = –0.96 and P < 0.0001 in 1999). No association was found in either year between the percentages of *M. graminicola* ascocarps and unidentified fruiting bodies (P = 0.84 in 1998 and P = 0.43 in 1999). In 2000, there was no significant association between the percentages of *M. graminicola* pycnidia or pseudothecia and that of unidentified fruiting bodies (P = 0.64 and 0.41, respectively). AUDPC was uncorrelated with percent unidentified fruiting bodies in 1998 (r = 0.15; P = 0.75) but positively correlated in 1999 (r = 0.75; P = 0.005).

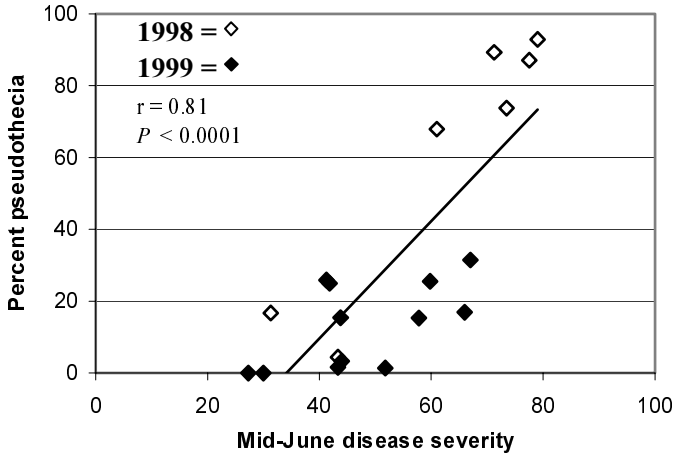

Fig. 2. Plot of pseudothecia as a percentage of all *Mycosphaerella graminicola* fruiting bodies versus disease severity assessed on 15 June 1998 and 17 June 1999 in four replicated field plots of 7 and 12 treatments, respectively; r and P values are from correlation analysis.

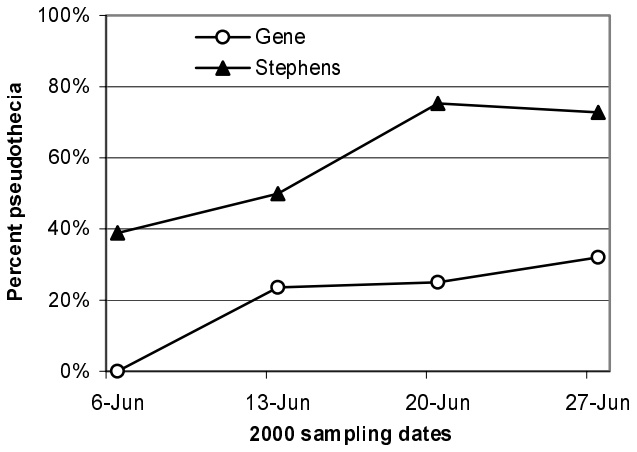

Fig. 3. Pseudothecia as a percentage of *Mycosphaerella graminicola* fruiting bodies sampled from leaves collected in field plots at 1-week intervals between 6 June and 27 June 2000 from winter wheat cvs. Gene and Stephens. Area under the disease progress curve (percentage-days) in the field plots was calculated as 788 and 2,185 for 'Gene' and 'Stephens', respectively, from assessments conducted on a whole-canopy basis eight times at 10- to 27-day intervals between 18 February and 6 June 2000.

DISCUSSION

We found a positive correlation between the susceptibility of host cultivars and the frequency of sexual reproduction by *M. graminicola*. Further, we found a positive association between the intensity of epidemics and the mean frequency of sexual fruiting bodies identified. In 1998 and 2000, the 2 years of high disease severity, 1.6 and 2 times as many pseudothecia as pycnidia were identified, respectively, while in 1999, the year of low disease severity, there were 7.4 times as many pycnidia as pseudothecia. (These data are from F-4 leaves, where the period available for sex is at or near its most protracted, and should not be taken as ratios for entire crops.) This fungus is probably heterothallic and bipolar (26), and thus presumably requires two individuals of opposite mating types to be in proximity in order to mate. It is therefore likely that the relatively higher infection densities found on susceptible cultivars and during intense epidemics lead to more frequent encounters between opposite mating types, which may explain the higher rate of sexual reproduction. Opposite mating types could come in contact where lesions abut; it has also been shown that different genotypes and mating types are often found within a single lesion (3,29,40).

Infection density could also affect the frequency of sex for reasons other than the likelihood of opposite mating-type encounters. Chamberlain and Ingram (8) noted that nutrient avail-

TABLE 3. *Mycosphaerella graminicola* pycnidial types in samples from field plots of winter wheat leaves at the ends of the 1998, 1999, and 2000 growing seasons

Treatment[a]	Percent[b]			Total number of pycnidia
	Microspores	Macrospores	Both	
1998				
Cashup	27.8	72.2	0.0	18
Lewjain	74.8	22.1	3.1	131
MacVicar	0.0	100.0	0.0	8
Madsen	25.0	62.5	12.5	8
Stephens	28.6	71.4	0.0	7
W-301	12.5	87.5	0.0	8
Stephens-spray[c]	10.0	90.0	0.0	10
Mean	25.5	72.3	2.2	27.1
1999				
Cashup	73.6	18.5	8.0	121
Foote	56.3	40.8	3.0	75
Gene	51.1	46.1	2.8	35
Lewjain	84.2	13.2	2.6	146
MacVicar	41.7	46.4	12.0	61
Madsen	44.3	52.1	3.7	73
Stephens	59.6	32.5	7.9	50
W-301	70.7	26.2	3.2	50
Stephens-high[c]	46.2	51.4	2.4	63
Stephens-low[c]	46.8	50.2	3.1	66
W-high	2.8	93.7	3.6	43
W-low	46.3	50.8	3.0	89
Mean	52.0	43.5	4.6	72.7
2000				
Gene	10.5	89.5	0.0	19
Stephens	43.8	50.0	6.3	16
Mean	27.2	69.8	3.2	17.5

[a] 'Cashup', 'Foote', 'Lewjain', and 'Madsen' were moderately resistant to *M. graminicola*. 'MacVicar', 'Stephens', and 'W-301' were susceptible. 'Gene' was susceptible in 1998 to 1999 and moderately resistant in 2000. By logistic regression, no effect of cultivar susceptibility on the presence of micropycnidiospores could be inferred due to significant rep×AUDPC interactions in both 1998 and 1999.

[b] Percentages of *M. graminicola* pycnidia containing only micropycnidiospores, only macropycnidiospores, or both.

[c] In 1998, chlorothalonil was applied to each plot at a rate of 0.84 liter per plot and a dosage of 3.7 ml of chlorothalonil per liter of water seven times during the growing season. In 1999, the dosages were 1.8 ml/liter (high) and 0.88 ml/liter (low) and five applications were made during the growing season.

ability can affect the switch between different reproductive processes, and suggested that increasing the density of single-spore inoculum enhances sexual reproduction. They summarized evidence that contact with mycelial extracts triggers sexual morphogenesis and/or the switch from vegetative growth to reproduction in facultatively sexual fungi, even across species lines.

It is also possible that certain genes conferring partial resistance in plant hosts exercise a pleiotropic effect on the frequency of sexual reproduction in their pathogens, e.g., by delaying the induction of sexual sporulation (8). However, the hypothesis that lower rates of sexual reproduction are a function of lesser disease severity, and not of resistance itself, is supported by the fact that, with one exception, fungicide applications reduced pseudothecial percentages on susceptible cultivars relative to unsprayed plots of the same cultivars. In the case of the 'W-301' high-spray treatment in 1999, the reason for the higher pseudothecial percentage is unclear.

Given the moderate negative correlation between days to heading and susceptibility to *M. graminicola*, the possibility must be considered that more frequent sexual reproduction is actually in some manner an outgrowth of more rapid progress to maturity. However, the lower percentages of pseudothecia in sprayed plots of 'Stephens' in 1998 and on 'Foote' in 1999, relative to other treatments with the same heading dates, argue against maturity as a significant confounding factor, as do the lower pseudothecial percentages on 'Gene' than on 'Stephens' in 2000.

Higher percentages of micropycnidiospores (microconidia) were found in 1999 than in the other 2 years. In some fungal genera, spermatia (microconidia) are born both in the same conidioma as macroconidia and in separate spermatium-bearing structures resembling the conidioma (11). However, it is unknown whether *M. graminicola* micropycnidiospores serve as spermatia, and whether they are as infective as macropycnidiospores. The higher percentages of micropycnidiospores found in 1999 would be consistent with a sexual role for these spores, because the epidemic that year was milder than those of 1998 and 2000, and fewer ascocarps had formed by the sampling date. It is also possible that micropycnidiospores are formed by *M. graminicola* in response to stress.

The negative correlation between the percentages of *M. graminicola* pycnidia and unidentified fruiting bodies found in 1998 and 1999, coupled with the lack of a similar correlation in the case of pseudothecia, suggests that the majority of the unidentified fruiting bodies were spent pycnidia. The absence of a linear relationship between either type of *M. graminicola* fruiting body and the unidentified fruiting bodies in 2000, together with the fact that the *Phaeosphaeria nodorum* epidemic was severe that year, is consistent with the hypothesis that a large fraction of the unidentified fruiting bodies in 2000 were spent pycnidia of *Phaeosphaeria nodorum*.

The higher frequency of unidentified fruiting bodies on more susceptible cultivars in 1999 may reflect the higher rate of pseudothecial formation on those cultivars, and could imply that immature pseudothecia account for a significant fraction of the unidentified fruiting bodies in 1999, when sexual reproduction appears to have been delayed by the mildness of the epidemic. The other possible explanations (that there is a higher rate of pycnidial or pseudothecial exhaustion on susceptible cultivars, or a lower rate of pycnidial formation) do not seem logical. There was also no evidence of a higher frequency of other pycnidial pathogens in 1999.

Why was 'Gene' susceptible to *M. graminicola* in 1999, yet moderately resistant in 2000? In 1995, after isolates had appeared with specific virulence to 'Gene' (13), the cultivar occupied 16% of the Willamette Valley winter wheat area, and consequently produced a significant share of total inoculum. Subsequently, the area planted to 'Gene' diminished by roughly 50% each year until it constituted 1.5% of the total wheat area in 1998 and 0.5% in 1999. Strains virulent to 'Gene' presumably were at increasingly lower frequencies during this period, and thus random events could at least partially account for the difference between the 2 years. In addition, competition from *Phaeosphaeria nodorum*, to which 'Gene' is susceptible, may have suppressed overall *M. graminicola* levels on 'Gene' in 2000. The unusually dry early spring of 2000 depressed Septoria tritici blotch levels until middle May and probably favored *Phaeosphaeria nodorum*, which apparently releases its ascospores later than *M. graminicola* (15) and is normally outcompeted in the Willamette Valley.

While our data do not disprove the suggestion of Kema et al. (26) that *M. graminicola* is capable of multiple sexual cycles in a season, they do suggest one or a very small number of sexual cycles per season in our environment. Relative to most other wheat-growing regions, the Willamette Valley of Oregon is highly conducive to severe *M. graminicola* epidemics and thus to the sexual stage of the fungus. In the 26 May 1999 samples from susceptible cvs. Gene and Stephens, no pseudothecia were found, although 1 month later, 5.2% of fruiting bodies from 'Gene' and 11.0% from 'Stephens' were *M. graminicola* pseudothecia. In the more severe epidemic of 2000, pseudothecial percentages for the first sampling date, 6 June, were 0.0% for 'Gene' and 12.1% for 'Stephens', and increased on both cultivars on the second and third sampling dates. It seems likely that the number of sexual cycles completed in a season is dependent on cultivar susceptibility, and that large numbers of pseudothecia are only produced toward the end of a growing season, in agreement with other findings (12,16,41,42).

It should be noted that fruiting body counts do not give direct information about the relative contributions of sexual and asexual reproduction to the genetic structure of the *M. graminicola* population. Empirical data about the respective multiplication efficiencies of pseudothecia and pycnidia (*sensu* Eriksen et al. [16]) under different environmental conditions are lacking. Molecular marker techniques are currently being used to address the question of how differing levels of host susceptibility affect the percentage of sexual descendants at the end of a growing season.

The effect of different levels of host resistance on the frequency of sexual reproduction in facultatively sexual parasites will be modulated by particular factors in each pathosystem. For example, *Strongyloides ratti*, the nematode parasite of rats, is parthenogenetic (18), whereas *M. graminicola* and *Phytophthora infestans* are both bipolar heterothallic fungi. For these plant pathogens, the frequency of sexual reproduction is probably strongly conditioned by the density of infections. In the case of *Phytophthora infestans*, a further limiting factor is the rapid tissue necrosis in susceptible cultivars, allowing insufficient time for oospores to form.

Hanson and Shattock (20) conclude that potato cultivars with very high levels of late blight resistance are likely to minimize formation of *Phytophthora infestans* oospores. Similarly, moderately resistant wheat cultivars limit sexual reproduction in *M. graminicola*, thus reducing the rate at which the pathogen can evolve to overcome host defenses or chemical controls.

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